



NEREUS.062A

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant : Hayashi et al.  
Appl. No. : 10/632,531  
Filed : August 1, 2003  
For : **DEHYDROPHENYLHISTINS  
AND ANALOGS THEREOF  
AND THE SYNTHESIS OF  
DEHYDROPHENYLHISTINS  
AND ANALOGS THEREOF**  
Examiner : Zachary C. Tucker

Group Art Unit 1624

I hereby certify that this correspondence and all marked attachments are being deposited with the United States Postal Service as first-class mail in an envelope addressed to: United States Patent and Trademark Office, P.O. Box 2327, Arlington, VA 22202, on

*March 3, 2006*  
(Date)

*Joseph M. Reisman*  
Joseph M. Reisman, Reg. No. 43,878

DECLARATION OF YOSHIO HAYASHI, Ph.D. SUBMITTED UNDER 37 C.F.R. § 1.132

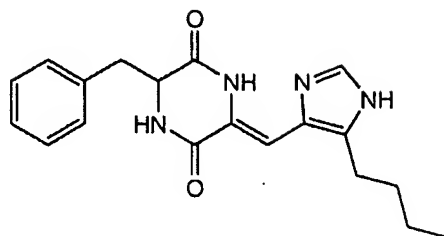
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Dear Sir:

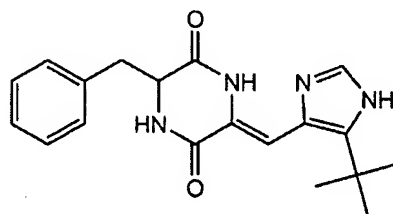
I, Yoshio Hayashi, Ph.D., do hereby declare:

1. I am currently an associate professor in the Department of Medicinal Chemistry at Kyoto Pharmaceutical University.
2. I am a co-inventor of the invention claimed in U.S. Application No. 10/632,531 and a paid consultant to the Assignee, Nereus Pharmaceuticals, Inc.
3. I have 23 years of experience in medicinal and organic chemistry.
4. The following experiment was carried out by me or under my direct supervision and control.
5. An assay of cytotoxicity against P-388 cells was conducted to determine IC50 values for n-butyl phenylahistin and tert-butyl phenylahistin. N-butyl phenylahistin has the chemical structure:

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Tert-butyl phenylahistin has the chemical structure:



6. The results, presented in the table below, indicate that tert-butyl phenylahistin has more than an order of magnitude greater cytotoxic activity than n-butyl phenylahistin.

Compound	IC <sub>50</sub> (μM) / P-388 Proliferation
n-butyl phenylahistin	11 ± 1.7
tert-butyl phenylahistin (S-form:R-form = 53:47)	0.83 ± 0.6
tert-butyl phenylahistin (S-form:R-form = 88:12)	0.26 ± 0.07

7. Based on the superior cytotoxic activity of tert-butyl phenylahistin with respect to n-butyl phenylahistin, as described in paragraph 6 above, I would also expect that tert-butyl dehydrophenylahistin would exhibit marked superiority over n-butyl dehydrophenylahistin. In addition, based on the data described in paragraph 6, I would also expect that other tert-butyl analogs of dehydrophenylahistin would have enhanced activity when compared to their n-butyl counterparts.

8. I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful, false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful, false statements may jeopardize the validity of the application or patent issuing therefrom.

Respectfully submitted,

Dated: March 2, 2006

By: 林 良雄  
Yoshio Hayashi, Ph.D.